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Production and characterization of biologically active Ala-Ser-(His)6-Ile-Glu-Gly-Arg-human prolactin (tag-hPRL) secreted in the periplasmic space of Escherichia coli.

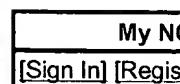
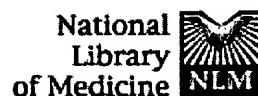
Morganti L, Huyer M, Gout PW, Bartolini P.

National Nuclear Energy Commission (IPEN-CNEN)-Cidade Universitaria, Sao Paulo, Brazil.

Human prolactin (hPRL) cDNA was obtained by screening of a pituitary cDNA library with a synthetic 21-mer oligonucleotide and with rat PRL cDNA. For its expression, use was made of a vector, p3SN8, containing tac-promoter-controlled sequences for a bacterial cellulase leader joined to sequences coding for Ala-Ser, a chromatographic affinity site consisting of six histidines and a Factor Xa cleavage site. The hPRL cDNA was inserted at the 3' end of the cleavage-site sequences. Expression in Escherichia coli led to secretion in the periplasmic space of a fully bioactive hPRL variant constituting authentic hPRL with a peptide tag, i.e. Ala-Ser-(His)6-Ile-Glu-Gly-Arg, at its N-terminal. This tag-hPRL could be rapidly and efficiently purified by metal-chelate affinity chromatography. The correct processing and quality of tag-hPRL was monitored by SDS/PAGE, Western-blot analysis, immunoassay and Nb2-lymphoma-cell bioassay. Treatment with Factor Xa for tag removal was only partially successful. Periplasmic secretion of tag-hPRL of the order of 0.7 micrograms/ml per A600 unit and one-step purification indicate feasibility for tag-hPRL production for in vitro diagnostic and research applications. This is the first report describing periplasmic secretion of a bioactive form of hPRL.

PMID: 8867898 [PubMed - indexed for MEDLINE]

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Synthesis and characterization of recombinant, authentic human prolactin secreted into the periplasmic space of *Escherichia coli*.

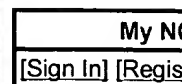
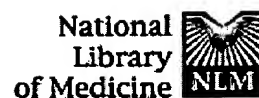
Morganti L, Soares CR, Affonso R, Gout PW, Bartolini P.

Biotechnology Department, National Nuclear Energy Commission (IPEN-CNEN), Cidade Universitaria, Sao Paulo, Brazil.

Recombinant, fully bioactive, authentic human prolactin (aut-hPRL) has been synthesized in transformed *Escherichia coli* HB2151 bacteria in a soluble, non-glycosylated form, which is secreted into the bacterial periplasm. Use was made of a bacterial expression vector, containing tac promoter-controlled sequences for the translation enhancer from bacteriophage T7 gene 10, and for a cellulase leader peptide from *Cellulomonas fimi* joined to sequences coding for aut-hPRL. This vector was derived from a previously described vector containing sequences of an hPRL variant, tag-hPRL (containing a 12-amino-acid peptide tag at the N-terminal end), using site-specific mutagenesis to delete the tag sequence. SDS/PAGE, partial N-terminal amino acid sequence analysis, Western blot analysis and Nb2 lymphoma cell in vitro bioassay indicated correct processing of the hormone. Periplasmic secretion of aut-hPRL, as measured by immunoassay, was relatively low (approx. 0.08 microgram/ml per A600 unit), in contrast to that of tag-hPRL which was approximately 8-fold higher, apparently a consequence of the tag sequence. This is the first report describing periplasmic secretion of biologically active, authentic hPRL.

PMID: 9477556 [PubMed - indexed for MEDLINE]

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Versatile epitope tagging vector for gene expression in mammalian cells.

Hosfield T, Lu Q.

Stratagene Cloning Systems, Inc., LaJolla, CA, USA.

We have constructed an epitope-tagging vector, pCMV-Tag1, for gene expression in mammalian cells. This vector, which allows for N-terminal, C-terminal and internal tagging of the gene product of interest with the FLAG and/or c-myc epitopes, enables researchers to rapidly and efficiently characterize gene products in vivo.

PMID: 9714892 [PubMed - indexed for MEDLINE]

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